GeneBio Systems, Inc.™

mRNA Capture Beads-Oligo (dT) Magnetic Beads

Cat. Number N401-01

Product Introduction

mRNA Capture Beads-Oligo (dT) Magnetic Beads have surface covalently conjugated with Oligo (dT), which base-pairs with Poly A at the tail of mRNA in eukaryotes. The Oligo (dT) Beads can efficiently separate complete and high purity mRNA from eukaryotic total RNA, animal and plant tissues or cell lysates. The isolated mRNA can be used in various molecular biological experiments: RT-PCR, solid phase cDNA library construction, RACE, Northern, etc.

Product Information	Oligo (dT) Beads（2.8 μm）	Oligo (dT) Beads （1 μm）
Oligo (dT) coupling capacity	~ 400 pmol/mg beads	~ 500 pmol/mg beads
mRNA binding capacity	1-2 μg/mg beads	
Bead concentration	5mg/mL	
Preservative solution	1×PBS, 0.01% Tween-20, 0.1% proclin-300	
Preservation condition	2~8℃	
Quality guarantee period	2 years	

Operating process

1. Preparation before use

1.1 Recommended buffers

The following are the recommended buffers. Users can adjust the salt concentration and detergent of the buffer according to the need.

Binding buffer	10 mM Tris-HCl (pH 7.5), 1.0 M NaCl, 1 mM EDTA
Lysis/binding buffer	100 mM Tris-HCl (pH 7.5), 500 mM NaCl, 10 mM EDTA, 1% SDS, 5 mM DTT
Washing buffer①	10 mM Tris-HCl (pH 7.5), 0.15 M NaCl, 1 mM EDTA, 0.1% SDS.
Washing buffer②	10 mM Tris-HCl (pH 7.5), 0.15 M NaCl, 1 mM EDTA
Elution Buffer	Nuclease-Free Water

Note: All reagents should be prepared with DEPC- treated purified water, and should be returned to room temperature before use. If the buffer precipitates, it can be preheated at 37℃ for 10min.

1.2 RNase-free 1.5mL centrifuge tube
1.3 Magnetic separator: an appropriate magnetic separator to separate the beads from a tube
1.4 Vortex generator
1.5 Rotating mixer
1.6 Pipette and tips

2. Wash the beads

2.1 Place the beads bottle on a vortex oscillator for 20 s to resuspend the beads. Pipette the desired volume of the beads into a new centrifuge tube. Add the same volume of binding buffer and vortex to resuspend the beads.
2.2 Put the centrifuge tube on a magnetic separator and placed for 1 min. Use a pipette to suck out the supernatant and remove the centrifuge tube from the magnetic separator.
2.3 Add the binding buffer with the same volume as the initial volume for later use.
Note: If mRNA is purified from total RNA, add half of the initial volume, and concentrate the magnetic beads to 10mg/mL.

3. Purification of mRNA from total RNA

3.1 For example, purify mRNA from 100 μg total RNA. Adjust 100 μg total RNA volume to 100μL with DEPC water.
3.2 Add the same volume of binding buffer (100μL) and mix by pipetting.
3.3 Heat at 65℃ for 2min to open the secondary structure, and then quickly place the tube on ice.
The 200μL of total RNA solution was added to 100μL washed magnetic beads. 1mg of washed magnetic beads dissolved in 100μL of binding buffer is used for every 100μg of total RNA (step 2). Mix well by pipetting.
3.4 Put it on a rotary mixer and incubate at room temperature for 10min.
3.5 Magnetically separate for 1min, and then carefully remove the supernatant. Remove the centrifuge tube from the magnetic separator.
3.6 Add 200μL of washing buffer②, and mix carefully by pipetting.
3.7 Magnetically separate for 1min, and carefully remove the supernatant. Remove the centrifuge tube from the magnetic separator.
3.8 Repeat the operation (3.6-3.7), washing twice in total.
Note: The beads should be completely resuspended for washing, and the clean supernatant should be removed as much as possible after the second washing.
3.9 According to the follow-up experiment, choose one of the following steps:
1) If mRNA does not need to be eluted from the magnetic beads, the magnetic beads should be washed again with the enzyme buffer of the downstream experiment.
2) If mRNA needs to be eluted from the magnetic beads: carefully remove the clean washing buffer ② (be careful not to aspirate the magnetic beads), then add 10–20μL of enzyme-free water or 10mM Tris-HCl (pH 7.5), blow and mix well, and incubate at room temperature for 2 minutes. Then, place the centrifuge tube on a magnetic separator, and transfer the supernatant containing mRNA to a new RNase-free centrifuge tube.
Note: The tube can be heated at 65℃-75℃ to improve the elution efficiency.

4. Isolation of mRNA from cell lysates

4.1 Wash the cell suspension with PBS, and centrifuge to obtain cell precipitate. Cell precipitate can be used immediately or stored at -80°C after freezing in liquid nitrogen.
4.2 Add 1 mL lysis/binding buffer to the cell pellet (1-4×10^6 cells). Repeat pipetting several times to ensure complete lysis. DNA released during lysis will cause the solution to become sticky, indicating that lysis is complete.
4.3 Reduce the viscosity by DNA cutting step. The lysate is processed 3 times through a 21-gauge needle using a 1-2 mL syringe. Repeated shearing may cause the lysate to foam, but foaming does not affect the mRNA yield. Foam can be reduced by centrifugation for 30s.
4.4 The lysate can be used for mRNA isolation immediately, or it can be frozen and stored at–80℃ for later use.
4.5 Magnetically separate the washed beads to remove the supernatant. Add the lysate and mix well.
4.6 Rotate and mix at room temperature for 5min for binding reaction. If the solution is viscous, increase the binding time.
4.7 Put the centrifuge tube on the magnetic separator for 1-2min, and remove the supernatant.
4.8 Wash the beads twice: once with 1 mL of washing buffer①, then once with 1 mL of washing buffer②.
Note: During washing, the beads should be completely resuspended, and the supernatant should be completely removed between washing steps.
4.9 According to the follow-up experiment, choose one of the following steps:
1) If the mRNA does not need to be eluted from the magnetic beads (such as the subsequent solid-phase cDNA synthesis), please wash it again with washing buffer②(500μL), and then wash it once with the enzyme buffer used in the downstream experiment.
2) Elute mRNA from magnetic beads: remove clean washing buffer②, and then add 10–20μL of enzyme-free water or 10 mM of Tris-HCl (pH 7.5). Incubate at 65°C -75°C for 2 minutes, then put the centrifuge tube on a magnetic separator, and quickly transfer the supernatant containing mRNA to a new RNase-free centrifuge tube.

Note: According to the abundance of mRNA, the final yield may be different among different tissues/cells. Generally, a mammalian cell contains about 10-30pg RNA, of which mRNA accounts for about 1-5%.

Note

1. Avoid freezing magnetic beads or other unnecessary operations.
2. In order to reduce the loss of magnetic beads, the time of magnetic separation should be no less than 1 min.
3. It is suggested that the extracted mRNA should be used for RT-PCR immediately. If preservation is needed, it is recommended to add RNase inhibitor to the eluent, elute the mRNA from the magnetic beads and freeze it.
4. All buffer and consumables used for mRNA extraction should be RNase-free.
5. The magnetic beads should be fully shaken and suspended evenly before removed from the magnetic beads storage tube. Bubbles should be avoided during operation.
6. If the purified rRNA residue is too much for downstream application, the second round of mRNA purification can be carried out.
7. It is recommended to use a good pipette tip and a reaction tube to avoid losses due to adhesion of magnetic beads and solution.
8. This product is for research use only.

Email: info@genebiosystems.com;
Phone: 1 833-LabShop;
Web: www.genebiosystems.com

GeneBio
Systems, Inc.™